STUDIES ON THE BIOLOGY OF SEWAGE DISPOSAL

A SURVEY OF THE BACTERIOLOGICAL FLORA OF A SEWAGE TREATMENT PLANT[1]

MARGARET HOTCHKISS

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INTRODUCTION

Of the many systems providing for sanitary sewage treatment each, in operation, has developed certain disadvantages for which local study and experience have tended to produce local remedies. Those systems which utilize biological factors have excited the interest of many students and the field offers a wealth of problems, upon the solution of which depends the successful operation and control of such sewage disposal plants as contain septic, Imhoff or activated sludge tanks and trickling or contact filter units. As a part of a more extended investigation to determine some of the conditions influencing biological factors a study of the bacterial population of the sewage disposal plant of Plainfield, North Plainfield and Dunellen was begun in November, 1923.

DESCRIPTION OF PLANT

In this plant the sewage passes through a Riensch Wurl screen, Imhoff tanks, sprinkling filter and final settling tank before it is discharged into a small brook. There are six Imhoff tanks, operated in units of three because experience has shown that the digestive efficiency of these tanks is increased by resting periods. After passing through the settling chambers of the Imhoff tanks the sewage, regulated by two dosing tanks, is sprayed upon one

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of two sprinkling filter beds. The sewage trickles over the stones and drains to a central gallery which leads to the final settling tank. At the time when these experiments were undertaken the effluent from this settling tank was discharged directly into a small brook.

The plant receives three to four million gallons of sewage per day and this represents about the capacity for which it was designed. The sewage is largely domestic waste and street washings are not included. When it reaches the plant it may be termed "stale sewage" as six hours are required for the material to reach the disposal plant after leaving the main sewer where it is collected.

OTHER INVESTIGATIONS

According to general opinion digestion tanks perform their action on sewage by the aid of anaerobic bacteria while aerobic bacteria act most vigorously in contact and trickling filter beds. The Imhoff tanks have for their function the separation and digestion of the solid particles in the incoming sewage and the digested particles form a fine mud which is drawn off on sand beds to dry. The sprinkling filter is designed "to change the putrescible organic matter of the sewage into stable forms, so that the effluent shall not be subject to putrefaction" (Worcester, 1912).

This separation of sewage treatment into two stages seems to have first been suggested by an English sanitary engineer, Scott-Moncrieff when, in 1891, he built a closed tank where anaerobic putrefaction was to take place with a series of trays containing coke for the second stage of nitrification (Metcalf and Eddy, 1916).

Notwithstanding the fact that anaerobic organisms have been considered important in putrefaction Winslow and Belcher (1904) conducting a survey of bacterial flora in stored sewage found few obligate anaerobes at any time. During the time of their experiments they noted a decrease in obligate aerobes while the facultative anaerobes, less sensitive to changes in oxygen tension, became the predominating organisms.

Most of the studies on sewage bacteria have been conducted under aerobic conditions due to the comparative simplicity of technique, and the results show that samples from sewage disposal plants contain an abundance of bacteria which are able to accomplish putrefactive changes under aerobic conditions. Such studies have been reported by Clark and Cage (1905) from the Lawrence Experiment Station, Massachusetts, by Kamm (1917) of the State Water Survey of Illinois and by Gaub (1923) at Rutgers College, New Jersey. The studies at the New Jersey Sewage Substation have not yet included the anaerobes but here also the work has shown an abundance of bacteria producing reduction and oxidation changes under aerobic conditions of culture.

The first work on the bacteria of the Plainfield disposal plant was undertaken in 1922–1923 by W. H. Gaub (1923) a graduate student at Rutgers College. During this investigation numerical determinations of bacteria in raw sewage and effluent of each unit were made daily for a period of a week, this work being done four times at intervals two months apart. Some organisms, able to grow on agar, were isolated and identified. The relative numbers of bacteria effecting certain reduction and oxidation changes were determined. From this work Gaub drew the following conclusions:

1. There was a continual decrease in number of bacteria in each unit of the plant.

2. The effluent of the plant caused an increase of bacteria in the small brook which received it but this increase was entirely eliminated in a distance of 450 feet.

3. Proteolytic bacteria and sulfate reducing bacteria were found in the effluents of all units. Nitrifying bacteria were distributed in Imhoff tank and sprinkling filter effluents with the greatest numbers in the latter effluent. Sulfur oxidizing bacteria were observed to be present especially in the effluents of the sprinkling filter and final settling tank. Cellulose decomposing bacteria were much less numerous than the other organisms studied.

EXPERIMENTAL RESULTS

The present paper represents work which is an amplification of the studies on the prevalence of the various organisms commenced by Gaub. Limitation of personnel and of equipment has prevented a complete and exhaustive study but it is believed that the results obtained may assist in the development of a knowledge of the bacteriology of sewage disposal plants.

The samples examined were collected from the influent to the Imhoff tanks after screening through the Riensch Wurl screen and from the effluent of the Imhoff tank unit. The scum and sludge and also the liquid 4 feet below the scum were examined as representative of the digestion chamber. Since one tank is not in constant operation the figures for this survey are based on data from three different tanks which were in operation at the time of sampling. These samples were collected twice a month for twelve months.

The samples from the sprinkling filter were analyzed once each month. These samples were collected from the surface spray and from the 1-, 3- and 5-foot levels of the bed by means of drain pipes placed for experimental use.

A series of dilutions (1:10, 1:100, 1:1000, 1:10,000, 1:100,000 and 1:1,000,000) was prepared from each sample. Several different media were then inoculated with one cubic centimeter of each dilution and after incubating at 20°C. the cultures were examined and by means of a series of simple chemical tests the dilution which marked the limit of various reactions was determined (Hotchkiss and Murray, 1923; New Jersey, 1923).

The media used to recognize the groups of bacteria were as follows:

1. Bouillon containing a cube of coagulated albumen. This was observed for liquefaction.
2. Bouillon containing ferric ammonium sulfate which was examined for a black precipitate of ferric sulfide as an indication of H_2S formation.
3. Inorganic sulfate medium to detect the formation of H_2S from inorganic material.
4. Thiosulfate medium for oxidation to sulfate.

5. Nitrate solution for the detection of nitrogen gas formation (denitrification).

6. Ammonium sulfate solution for detection of nitrite formation.

7. Nitrite solution for the detection of nitrate formation. It will be observed that 1 and 2 record proteolytic changes, 2, 3, 5 represent reduction processes and 4, 6 and 7 represent oxidation processes. The bacteriological activities studied were therefore concerned with proteolysis and with sulfur and nitrogen transformations. The groups of bacteria producing these changes were universally present and may be assumed to produce similar physiological changes in the sewage during treatment.

TABLE 1

Arithmetical averages. Thousands of bacteria per cubic centimeter throughout sewage plant

BACTERIA	INFLUENT	IMHOFF TANK DIGESTIVE CHAMBER	EFFLUENT	FILTER	FILTER EFFLUENT	FINAL EFFLUENT
Nitrate reducers	278.2	727.7	248.7	208.4	16.2	190.0
H_2S producers (protein)	83.4	424.0	131.9	172.9	2.4	160.1
Albumen digesters	123.9	244.0	60.4	102.6	17.4	135.8
H_2S producers (sulfate)	3.6	215.5	17.0	19.1	0.36	2.4
Ammonium salts to nitrites	43.9	73.0	9.2	39.8	1.3	0.4
Nitrites to nitrates	87.6	83.8	48.3	53.3	12.7	23.4
Thio sulfite to sulfate	2.2	27.8	0.3	2.0	12.8	1.43

The data represented determinations on 20 to 24 samples collected throughout the year from the influent, from various portions of the digestive chamber and from the effluent of the Imhoff tanks. Data on 12 samples each from the various layers of the filter bed was obtained. Data were available on 9 to 11 samples of the final effluent. The maximum number of determinations on the filter effluent was 10, made for the denitrifying organisms and the minimum was 5 determinations made on organisms producing H_2S from protein. The other figures are based on 9 determinations.

The data were first grouped by arithmetical averages as in table 1, which shows the general relationships of the various bacteria. It is seen that nitrate reducing and proteolytic organisms predominate in the Imhoff tank and sprinkling filter. It is noteworthy that the nitrifying bacteria, which require oxygen and which have been noted as being sensitive to organic material are distributed throughout the plant being present in samples from the Imhoff tank as well as in the sprinkling filter. In the Imhoff tank the nitrifying bacteria do not produce sufficient change to make it possible to detect nitrites or nitrates directly in samples where the bacteria can be demonstrated.

The dilution method of computing bacterial numbers inevitably gives large fluctuations. An attempt to avoid the errors of extreme variation was made by determining the mode of the

TABLE 2

Seven groups of bacteria showing numerical value per cubic centimeter which occurred most frequently

BACTERIA	INFLUENT	DIGESTION CHAMBER	EFFLUENT	FILTER
Nitrate reducers	100,000	1,000,000	100,000	100,000
H_2S producers (protein)	10,000	100,000	10,000	10,000
Albumen digesters	10,000	100,000	1,000	1,000
H_2S producers (sulfate)	1,000	100,000	10,000	10,000
Ammonium salts to nitrites	100	1,000	1,000	1,000
Nitrites to nitrates	0	1,000	0	1,000
Thio sulfite to sulfate	100	10,000	100	1,000

different bacterial groups. This grouping is presented in table 2 and it shows the greatest frequency with which the different groups of bacteria occurred. In it are presented the relative abundance of the bacteria and the general concentration in the digestion chamber of the Imhoff tank as compared with the sprinkling filter is seen. The nitrate reducers are the most numerous in each location, the oxidizing organisms are the least numerous while the proteolytic and hydrogen sulfide producers occupy an intermediate position.

Another statistical arrangement has also been tested, perhaps better fitted to the expression of the figures obtained. This is the geometric average used by Jevons (1883). The geometric average minimizes the effect of occasional large figures. It is obtained by multiplying the n items of each series and extracting the nth root of the product. Table 3 represents averages obtained by this method using the same data as that on which table 1 is based. A comparison of table 1 and 3 shows the lower figures obtained in the geometric average.

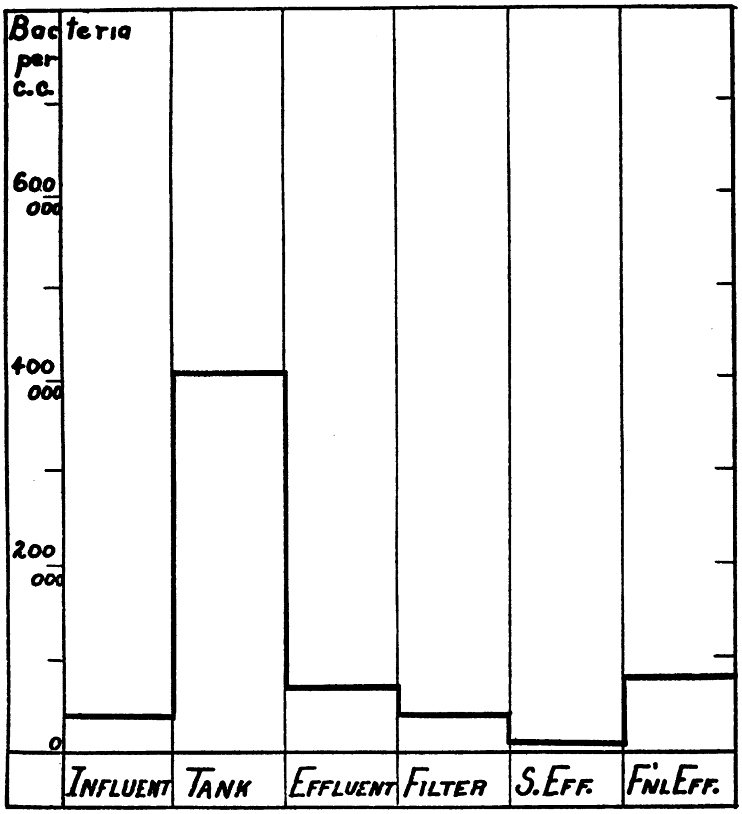

FIG. 1. NUMBER OF BACTERIA, REPRESENTING THE TOTAL OF SEVEN GROUPS, FOUND THROUGHOUT SEWAGE PLANT

The general statements made as to the relative predominance of the organisms are borne out by table 3. All tables show that the digestive chamber of the Imhoff tank contained the greatest concentration of bacteria per cubic centimeter. There were fewer bacteria per cubic centimeter in the effluent from the sprinkling filter than elsewhere in the plant. It must be remem-

TABLE 3

Geometrical averages. Bacteria per cubic centimeter throughout sewage plant

BACTERIA	INFLUENT	IMHOFF TANK DIGESTIVE CHAMBER	EFFLUENT	FILTER	FILTER EFFLUENT	FINAL EFFLUENT
Nitrate reducers	28,480	223,040	64,494	22,456	5,011	74,989
H_2S producers (protein)	10,000	140,154	10,000	9,151	630	5,179
Albumen digesters	3,368	19,419	2,993	9,363	1,930	5,994
H_2S producers (sulfate)	730	21,174	900	1,132	59	100
Ammonium salts to nitrites	332	3,007	367	1,128	59	166
Nitrites to nitrates	74	1,811	22	194	77	215
Thio sulfite to sulfate	316	2,243	53	175	237	158
Total	43,300	410,848	78,829	43,599	8,003	86.801

TABLE 4

Geometrical averages. Bacteria per cubic centimeter in three levels of digestive chamber of Imhoff tank

BACTERIA	SCUM	LIQUID	SLUDGE
Nitrate reducers	480,637	151,999	151,999
H_2S producers (protein)	263,664	87,992	100,000
Albumen digesters	12,452	8,858	59,519
H_2S producers (sulfate)	7,305	15,199	13,688
Ammonium salts to nitrites	6,061	1,492	4,328
Nitrites to nitrates	2,080	592	904
Thio sulfite to sulfate	2,565	299	811
Total	774,764	266,431	341,249

TABLE 5

Geometrical averages. Bacteria per cubic centimeter in three levels of filter bed

BACTERIA	PIPE I	PIPE II	PIPE III
Nitrate reducers	12,338	28,942	28,942
H_2S producers (protein)	16,681	5,994	5,994
Albumen digesters	2,310	3,831	6,812
H_2S producers (sulfate)	681	2,610	837
Ammonium salts to nitrites	383	2,424	1,425
Nitrites to nitrates	68	242	412
Thio sulfite to sulfate	177	209	146
Total	**32,638**	**44,252**	**44,567**

bered that this average would be raised if many samples were collected at the times of spring and fall slough, when the film on the filter stones becomes loosened and passes out with the effluent liquid. It is also noteworthy that after passing through the final settling tank the number of organisms increases. Figure 1 presents these facts graphically.

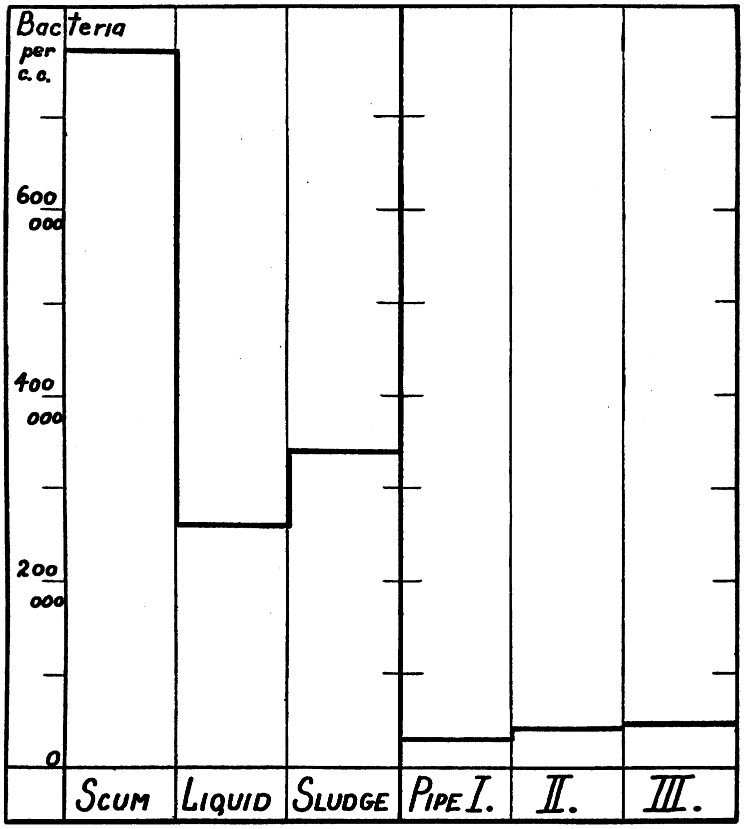

FIG. 2. NUMBER OF BACTERIA, REPRESENTING THE TOTAL OF SEVEN GROUPS, FOUND IN THREE LEVELS OF THE IMHOFF TANK AND THE SPRINKLING FILTER

Tables 4 and 5 show the averages for the data on the samples collected from the digestion chamber of the Imhoff tank and the sprinkling filter bed. The items "scum," "liquid" and "sludge" are used to designate samples taken from the surface layer of the gas vents, from the material 4 feet below the surface and from material drawn from the sludge pipe which taps the tank at its lower level. The scum is composed of fat, grease and such solid particles as have been brought to the surface attached to gas bubbles, which because of lesser density float on the liquid. Its bacterial content is higher than that of liquid or sludge prob-

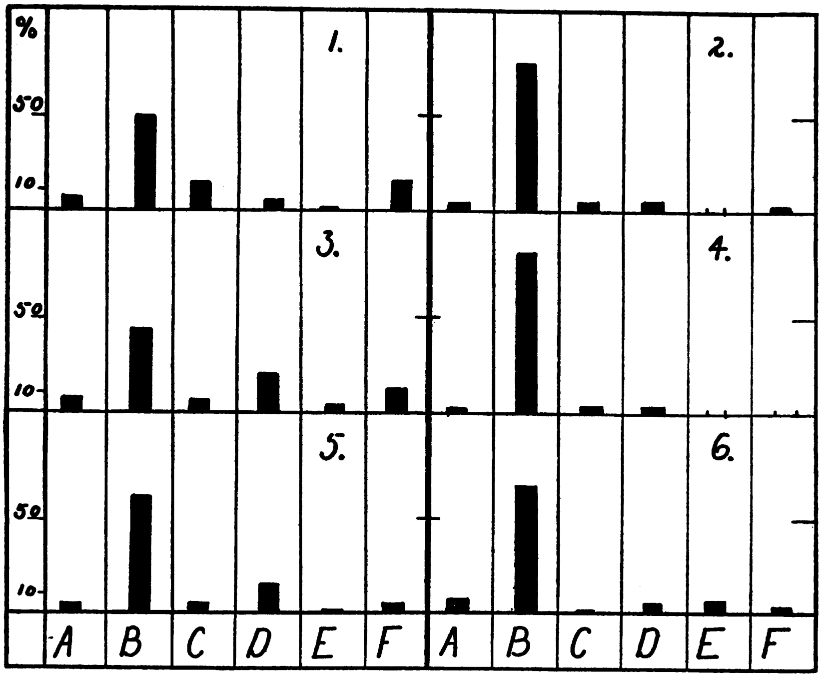

FIG. 3. PERCENTAGE OF BACTERIA PER CUBIC CENTIMETER THROUGHOUT SEWAGE PLANT

A, influent; *B*, digestion chamber; *C*, effluent Imhoff tank; *D*, filter; *E*, sprinkling filter; *F*, final effluent. *1*, nitrate reducers; *2*, H_2S producers (protein) *3*, albumen digesters; *4*, H_2S producers (sulfate); *5*, total nitrifiers; *6*, sulfur oxidizers.

ably for much the same reasons that give cream a higher bacterial count than the milk below it.

The sewage which drains through the filter bed has a smaller concentration of bacteria per cubic centimeter but there is a slight increase in bacterial content at the bottom of the bed (fig. 2). This is probably due to contact with the film which surrounds the stones and which is the locus for a complex animal and plant population. Bacteriological studies were also made on this film and the results will be presented in a later paper.

Although the digestion chamber contains the greatest number of bacteria per cubic centimeter no striking selective action in the types of bacteria which compose the flora was observed. Figure 3 expresses the percentage of bacteria of each group throughout the sewage plant. Whether oxidizing or reducing bacteria, the highest percentage is found in the digestion chamber. This merely shows the straining action of the tank for as the sewage passes through the settling chamber the solids sink and find their way through the slots into the digestion chamber carrying bacteria with them. Hence the material in the tank becomes more and more concentrated. However, conditions in the tank are not actually hostile to any of these groups of organisms as is witnessed by the fact that the nitrifying and sulfur oxidizing groups are not eliminated.

Does the complexion of the flora change markedly in any of the units of the plant? Figure 4 shows the relative percentage of the groups under consideration in each unit of the sewage system. The bacteria which reduce nitrate to nitrogen gas are most numerous throughout the plant. This is a change which is considered to take place under conditions of insufficient aeration (Marshall, 1921) and it is noteworthy that passage through an Imhoff or settling tank increases the percentage of these organisms. Next to the nitrate reducing organisms in numerical importance are the producers of hydrogen sulfide from protein. These are abundant in the influent, the digestion chamber and the sprinkling filter. Their numbers are diminished as the sewage passes through the plant until they make up about 7 per cent in the filter effluent. This low percentage is not influenced by the passage of the sewage through the final settling tank.

Another numerous group of organisms is the group of albumen digesters. It might be supposed that this group of proteolytic organisms would be most abundant in the Imhoff tank where protein digestion presumably takes place but rather contrary to expectation the percentage is highest in the sprinkling filter. The

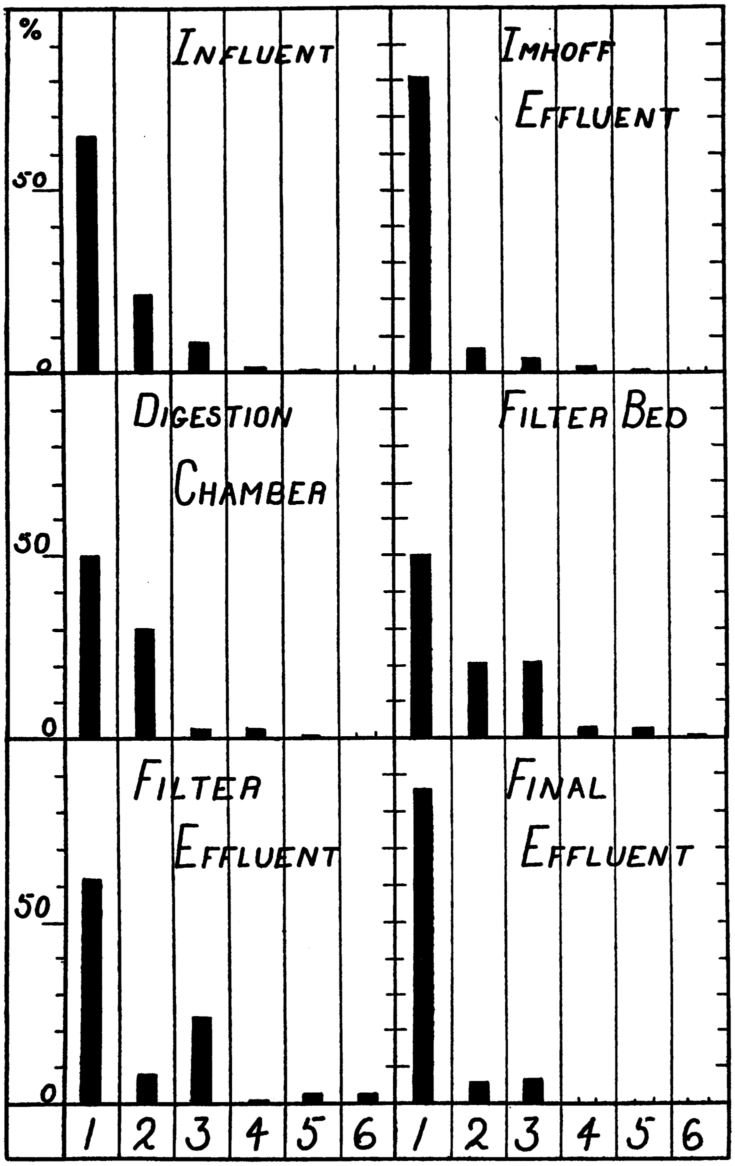

FIG. 4. PERCENTAGE OF BACTERIA PER CUBIC CENTIMETERS IN EACH UNIT OF SEWAGE PLANT

1, nitrate reducers; *2*, H_2S producers (protein); *3*, albumen digesters; *4*, H_2S producers (sulfate); *5*, total nitrifiers; *6*, sulfur oxidizers.

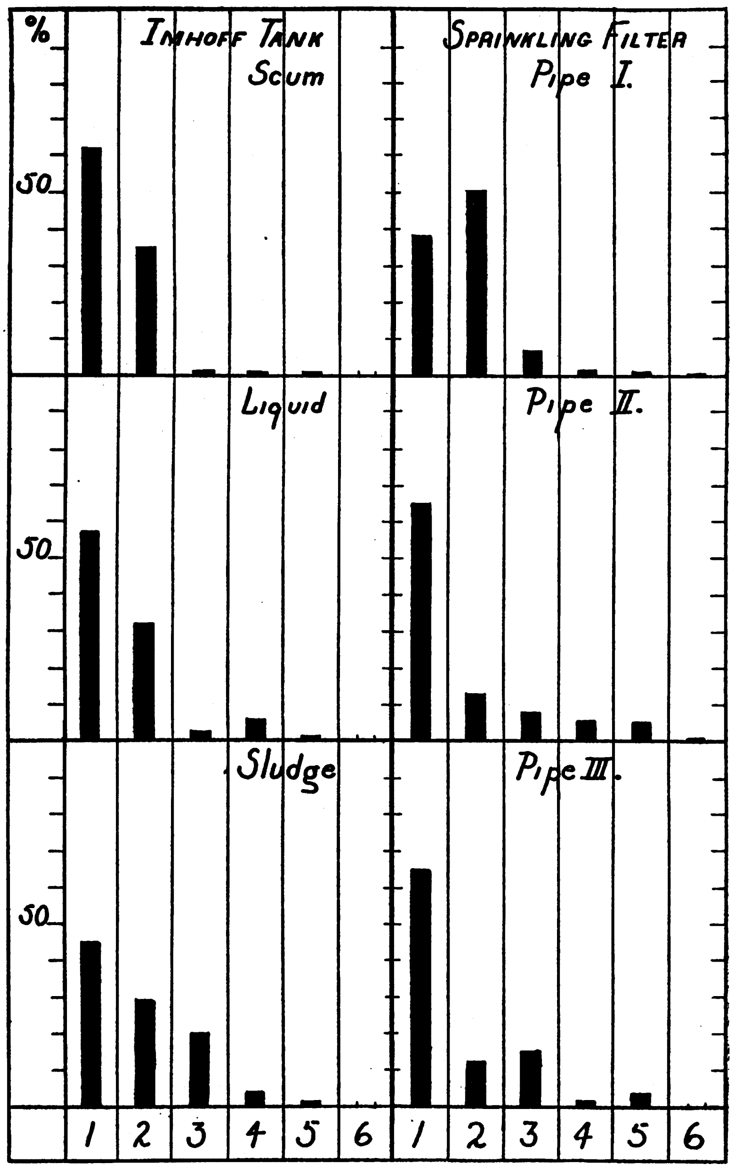

FIG. 5. PERCENTAGE OF BACTERIA PER CUBIC CENTIMETER IN SAMPLES FROM THREE LEVELS OF DIGESTION CHAMBER (IMHOFF TANK) AND SPRINKLING FILTER

1, nitrate reducers; *2*, H_2S producers (protein); *3*, albumen digesters; *4*, H_2S producers (sulfate); *5*, total nitrifiers; *6*, sulfur oxidizers.

actual numbers here average about one-half those found in the digestion chamber.

Both the nitrogen and sulfur oxidizers averaged a barely considerable percentage of organisms in any sample. This percentage was highest in the sprinkling filter and the filter effluent. It is to be noted that even this slight increase was lost after the sewage passed through the settling tank.

No striking change of flora has therefore been demonstrated as the sewage passes through the treatment plant. Some oxidizing bacteria are found in the Imhoff tank which presumably favors anaerobic changes and there is no striking increase in the percentage of oxidizing bacteria in the filter bed. On the other hand throughout the plant there is a decided decrease in the relative percentage of those bacteria which produce hydrogen sulfide.

Figure 5 shows the composition of the material from various parts of the Imhoff tank and the three levels in the filter. It must be remembered that the digestion chamber contains many more bacteria per cubic centimeter than the liquid which finds its way through the filter. In the digestion chamber nitrate reducers and hydrogen sulfide producers predominate slightly in the scum and liquid. Albumen digesters become more numerous in the sludge. In general the proportion of organisms is the same, for the liquid and sludge are agitated by gas evolution and the contents of the chamber become mixed.

In the filter bed a change suggestive of decreasing oxygen tension is seen. The nitrate reducing organisms occupy a larger place as the samples are secured deeper in the bed. The albumen digesters also increase and the numbers in the lower levels seem to influence the character of the effluent. These organisms make up 7 and 8 per cent of the bacteria found in the first and second pipes which are 1 and 3 feet from the surface of the bed and 15 per cent of the bacteria in the third pipe which is 5 feet below the surface while the effluent which drains from the bed contains 24 per cent of these organisms. In contrast to the increase in this type of proteolytic organism in the filter bed it seems that the flow of sewage through an Imhoff or settling tank does not increase the predominance of the albumen digesters. The organisms form 7 per cent of the influent and 4 per cent of the effluent of the Imhoff tank. They have increased to 24 per cent in the filter effluent and yet the final effluent contains only 6 per cent. Perhaps these organisms find their most favorable habitat around undissolved protein material which tends to sink and so to rid the liquid of the proteolytic organisms.

The filter bed has been regarded as the site of oxidation changes which tend to render the effluent stable and inoffensive. If the sulfur cycle is considered it will be noted that the producers of hydrogen sulfide from protein which form 51 per cent of the bacteria in the filter influent from the top layer of the filter bed decrease until there remain only 7 per cent in the filter effluent. The group of organisms which produce hydrogen sulfide from inorganic sulfates are less numerous. They form 2 per cent of the bacteria in the liquid of the upper level of the filter bed, increase to 5 per cent in the middle layer, decrease to 1.8 per cent in the lower layer while the filter effluent contains 0.7 per cent. In other words these hydrogen sulfide producers tend to be eliminated also. Turning to the sulfur *oxidizing* bacteria, those which grew under our cultural conditions formed only 0.5 to 0.3 per cent of the bacteria in the liquid from the filter bed; however, the effluent from the filter bed contains 3 per cent and this indicates an increase of these organisms. It is, then, the tendency of hydrogen sulfide producers in the filter bed to decrease in numbers but the sulfur oxidizing bacteria increase. In spite of such a tendency the oxidizers do not increase to such an extent that they become of great numerical importance.

In considering the nitrogen cycle it will be remembered that the nitrifying organisms are divided into two groups—those which produce nitrite from ammonium salts and those which utilize the nitrite and form nitrates in so doing. The nitrite producers are here the more numerous group. They form 1 per cent of the organisms in the liquid from the upper layer of the filter bed, 5 per cent in the second layer, 3 per cent in the third layer and 0.7 per cent in the effluent. The nitrate producers form only 0.2 per cent of the bacteria in the liquid from the upper layer, 0.4 per cent in the second layer and 0.9 per cent in the third layer. The effluent contains 1 per cent.

Chemical analyses made by Rudolfs and Campbell (New Jersey, 1923) during the same period show the same story. As table 6 shows, both the nitrate and the nitrite nitrogen increase as the lower levels of the filter bed are reached. Other workers have noted that the depth of the filter bed increases the nitrate nitrogen (Kinnicutt, Winslow and Pratt, 1919).

In spite of the increase in nitrate nitrogen in the filter bed our bacteriological studies show that the nitrate reducing bacteria are predominant. These organisms form 50 per cent of the bacteria in a cubic centimeter of filter liquid, while the nitrifying

TABLE 6

Average nitrite and nitrate nitrogen in liquid from different levels of the sprinkling filter bed, expressed in parts per million (August, 1922, to June, 1923)*

FILTER BED	NO_2-N	NO_3-N
Surface	0.133	0.511
First pipe	0.130	0.494
Second pipe	0.191	1.400
Third pipe	0.213	3.901
Effluent	0.280	5.665

*Compiled from table 13, Bulletin 390, New Jersey Agricultural Experiment Station.

organisms form 3 per cent of the bacterial population in the same location. The filter effluent contains 62 per cent nitrate reducers and 1.7 per cent nitrifiers. The liquid from the digestion chamber contains 57 per cent of nitrate reducers and 0.78 per cent of nitrifiers. The ratio of the nitrifiers to the nitrate reducers in the three locations is 0.059, 0.027, 0.013. Therefore it seems that conditions in the filter bed are such that the nitrifying organisms multiply more rapidly than in other parts of the disposal plant. It has been suggested (New Jersey, 1923) that one factor is the partial digestion of organic matter to simpler forms which are more readily attacked by the nitrifying organisms. This may account for the fact that nitrification is greater in the lower levels of the bed although a priori it would seem that oxygen tension deeper in the bed would be less favorable for oxidation changes.

SUMMARY

By the use of the dilution method using different media the bacterial population in the sewage disposal plant under consideration was divided into arbitrary groups according to physiological activities produced. The groups here discussed are

1. Organisms reducing nitrate to nitrogen gas.
2. Organisms splitting protein with the production of hydrogen sulfide.
3. Organisms liquefying coagulated egg albumin.
4. Organisms reducing inorganic sulfates to hydrogen sulfide.
5. Organisms oxidizing ammonium salts to nitrites.
6. Organisms oxidizing nitrites to nitrates.
7. Organisms oxidizing thio sulfite to sulfate.

In this preliminary survey of the bacterial population no attempt was made to isolate and observe individual bacterial types.

The study was carried on for one year and conclusions are based on the geometrical averages of the data obtained.

The largest number of bacteria per cubic centimeter were found in the digestive chamber of the Imhoff tank. This was true whether organisms of a reducing or an oxidizing type were considered. The effluent from the sprinkling filter, except at the time of slough, contained the fewest bacteria per cubic centimeter.

Of the groups considered, the organisms most important numerically throughout the plant were the nitrate reducers, the hydrogen sulfide producers (from protein) and the albumen digesters. Nitrifying and sulfur oxidizing bacteria occurred throughout the plant, and were consistently found even in the digestion chamber of the Imhoff tank. Chemical analyses of material from the digestion chamber, however, showed no nitrites or nitrates. The number of nitrifying organisms increased in the filter bed although they never became numerically predominant and higher percentages of nitrifiers were found in liquid which had trickled through the lower levels of the bed than that collected near the surface.

An interesting fact was the drop in the numbers of hydrogen sulfide producing organisms as the sewage passed through the plant. This was the most striking change in the bacterial flora which could be demonstrated.

In general it may be said that in a sewage plant handling "stale" sewage in a quantity at about its capacity the proteolytic and reducing organisms overbalance the oxidizing organisms, however the practical results obtained at the plant under examination show that by careful operation a satisfactory effluent can nevertheless be obtained.

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